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ORIGINAL ARTICLE

Expression of NAD(P)H quinone dehydrogenase 1 (NQO1) is increased in the endometrium of women with endometrial cancer and women with polycystic ovary syndrome

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[Correction added on 25 October 2017 after first online publication: The surname "Fuentes-Utrilla" was changed to "Fuentes-Utrilla".]

Summary

Objective: Women with a prior history of polycystic ovary syndrome (PCOS) have an increased risk of endometrial cancer (EC).

Aim: To investigate whether the endometrium of women with PCOS possesses gene expression changes similar to those found in EC.

Design and Methods: Patients with EC, PCOS and control women unaffected by either PCOS or EC were recruited into a cross-sectional study at the Nottingham University Hospital, UK. For RNA sequencing, representative individual endometrial biopsies were obtained from women with EC, PCOS and a woman unaffected by PCOS or EC. Expression of a subset of differentially expressed genes identified by RNA sequencing, including NAD(P)H quinone dehydrogenase 1 (NQO1), was validated by quantitative reverse transcriptase PCR validation (n = 76) and in the cancer genome atlas UCEC (uterine corpus endometrioid carcinoma) RNA sequencing data set

($n = 381$). The expression of NQO1 was validated by immunohistochemistry in EC samples from a separate cohort ($n = 91$) comprised of consecutive patients who underwent hysterectomy at St Mary's Hospital, Manchester, between 2011 and 2013. A further 6 postmenopausal women with histologically normal endometrium who underwent hysterectomy for genital prolapse were also included. Informed consent and local ethics approval were obtained for the study.

Results: We show for the first time that NQO1 expression is significantly increased in the endometrium of women with PCOS and EC. Immunohistochemistry confirms significantly increased NQO1 protein expression in EC relative to nonmalignant endometrial tissue ($P < .0001$).

Conclusions: The results obtained here support a previously unrecognized molecular link between PCOS and EC involving NQO1.

KEYWORDS

endometrial cancer, endometrium, NQO1, polycystic ovary syndrome

1 | INTRODUCTION

Endometrial cancer (EC) is the most common gynaecological cancer affecting women in the United States, with an estimated 60 050 new cases in 2016.¹ The incidence of EC has increased by over 65% since the late 1970s correlating with rising incidence of obesity and increased longevity.^{2,3} EC is usually treated by hysterectomy, but surgery carries increased risk in obese women and renders premenopausal women infertile. In addition to its negative impact on quality of life, EC poses a significant economic burden on health services.

Polycystic ovary syndrome (PCOS) is the commonest female endocrinopathy affecting 3%-20% of women of reproductive age.^{4,5} Women with PCOS experience obesity, infrequent menstrual periods, infertility, excess systemic androgens, insulin resistance and hirsutism, and have enlarged ovaries with multiple small follicles on ultrasound imaging.⁶ Women with PCOS have an increased risk of type 2 diabetes in later life and a threefold to fourfold increased risk of EC.^{7,8}

The exact mechanisms that predispose PCOS women to EC remain unknown. Current hypotheses include a link between obesity and elevated oestrogen levels, inflammation, type 2 diabetes and hyperinsulinaemia.⁹ In recently published studies, we found altered expression of genes involved in insulin signalling (IGF-1, IGFBP1 and PTEN) and lipogenic gene regulation in the endometrium and serum of women with PCOS and EC compared with controls.^{10,11} Genes related to immunoregulation/inflammation,¹² antioxidants⁹ and impaired progesterone-mediated decidualization¹³ have also been suggested as possible mechanisms linking PCOS and EC.

This complexity highlights the need to characterize the transcriptome of the endometrium of women with PCOS to advance understanding of mechanisms linking PCOS and EC. To our knowledge, comparative transcriptomic, proteomic or metabolomic studies of patients with PCOS and EC are as yet unavailable. The aim of this proof of principle study was to perform comparative RNA sequencing

profiling of endometrial biopsies from women with PCOS and EC and validate these findings in a large cohort of patients with EC.

2 | METHODS

2.1 | Study design, patient recruitment, sample acquisition and processing

Details of the methods used in patient recruitment for the Nottingham cohort have been previously described in detail elsewhere.^{10,11} Briefly, patients ($N = 76$) were recruited into to a cross-sectional study conducted within the division of Obstetrics and Gynaecology and Child Health, at Nottingham University Hospital in the United Kingdom. Participants were prospectively recruited from July 2013 to February 2014. Research ethics approval was obtained from the National Research Ethics Service, East Midlands-Northampton committee (13/EM/0119) prior to commencement of recruitment. The project was also reviewed and approved by the relevant local ethics committees at the University of Nottingham. The Helsinki Declaration was strictly observed. Informed consent was obtained from all participants. For RNAseq, representative endometrial biopsies were obtained from individual women from each arm, specifically with EC (age 43, BMI = 35.9), polycystic ovary syndrome (age = 41, BMI = 35.9) and an age- and BMI-matched women unaffected by PCOS or EC (age = 42, BMI 32.01) and stored immediately in RNAlater (Sigma-Aldrich, Gillingham, UK). For qRT-PCR validation, participants were recruited in three arms: PCOS ($n = 26$), EC ($n = 25$) and control ($n = 25$). The participants were between 19 and 84 years of age and not on any hormonal treatment. Pregnancy was excluded prior to the recruitment using standard urine pregnancy tests. The EC group consisted of women with histopathologically proven endometrioid (type 1) adenocarcinoma of the endometrium undergoing total hysterectomy (by laparotomy or laparoscopically) who had not received previous neo-adjuvant chemo- or radiotherapy. Women were excluded from the study for prior history of papillary serous

TABLE 1 Participants' characteristic, biochemical and hormonal data of the Nottingham cohort. One-way ANOVA test was used to determine the difference between the groups

	Control (n = 25)	PCOS (n = 26)	Endometrial cancer (n = 25)	P value
Age (years); Mean (SD)	45.96 (13.34)	31.88 (5.975)	62.64 (11.10)	<.0001*
BMI (Kg/m ²); Mean (SD)	29.27 (2.467)	29.60 (3.116)	33.12 (5.959)	.0021*
WHC ratio; Mean (SD)	88.56 (3.241)	88.5 (3.992)	96.86 (12.99)	.0003*
Systolic BP(mm Hg); Mean (SD)	135.9 (8.729)	134.1 (7.591)	148.2 (11.42)	<.0001*
Diastolic BP (mm Hg); Mean (SD)	81.88 (7.563)	83.35 (7.746)	84.80 (7.455)	.4012
Fasting insulin; Mean (SD)	13.47 (6.002)	20.04 (31.79)	18.3 (16.26)	.5199
Fasting glucose; Mean (SD)	4.844 (0.4788)	5.142 (0.8363)	6.4 (1.649)	<.0001*
HOMA-IR; Mean (SD)	0.1809 (0.1065)	0.2617 (0.4405)	0.3139 (0.3766)	.3871
LDL; Mean (SD)	2.820 (0.8539)	2.738 (0.8174)	2.632 (0.9919)	.7561
HDL; Mean (SD)	1.5 (0.3136)	1.415 (0.2664)	1.648 (0.3754)	.0381*
TG; Mean (SD)	1.344 (0.6752)	1.373 (0.4609)	1.54 (0.5694)	.4305
Total cholesterol; Mean (SD)	5.004 (0.9654)	1.373 (0.4609)	1.54 (0.5694)	.5293
FSH; Mean (SD)	17.88 (24.81)	5.008 (2.788)	49.01 (20.83)	<.0001*
LH; Mean (SD)	12.95 (12.04)	12.31 (11.08)	28.64 (13.08)	<.0001*
Testosterone; Mean (SD)	1.516 (0.6309)	2.846 (0.7089)	1.492 (0.8684)	<.0001*
Oestradiol; Mean (SD)	414.5 (655.8)	331.9 (261.3)	96.72 (55.39)	.0204*
Progesterone; Mean (SD)	6.448 (15.37)	11.2 (14.54)	1.28 (0.5292)	.0192*
SHBG; Mean (SD)	55.36 (38.65)	34.46 (14.03)	50.6 (17.01)	.0120*

*P value <.5 is significant are indicated.

adenocarcinoma or metachronous cancers of the ovary, endometrium or cervix. The PCOS cohort was defined using the Rotterdam European Society for Human Reproduction and Embryology (ESHRE) and the American Society of Reproductive Medicine (ASRM) criteria. Baseline demographic details, blood pressure, weight and body mass index were calculated (kg/m²), the Ferriman Gallwey hirsutism score was recorded, and hip-waist circumference was recorded (cm) with participants wearing indoor clothing. The control group comprised of healthy women without EC or PCOS, not on any hormonal therapy, undergoing pelvic surgery for benign indications. The following clinical parameters were measured in participants using standard UK National Health Service services: fasting blood glucose, low-density lipoprotein, high-density lipoprotein, triglycerides, sex hormone binding globulin, testosterone, follicle stimulating hormone, luteinizing hormone, prolactin, 17-hydroxy-progesterone and thyroid function. A Pipelle® endometrial catheter was used to biopsy endometrial tissue, and samples were snap frozen at -80 C for subsequent processing. Patient characteristics and the results of endocrine and metabolic assays were as previously reported (Table 1).

2.2 | RNA sequencing (RNAseq) and quantitative reverse transcriptase PCR (qRT-PCR) analysis of patient endometrial samples

Total RNA was isolated using an RNeasy extraction kit, with on-column DNase digestion (Qiagen, Manchester, UK). RNA quality (RIN > 7) was confirmed using an Agilent bioanalyser. Samples were submitted to Edinburgh Genomics for library preparation and analysed using

an Illumina HiSeq platform using standard protocols. For quantitative reverse transcriptase PCR validation of RNAseq results, the samples were a subset (Table 1) of the patients described previously.¹⁰ The expression of identified differentially expressed genes was examined in the cancer genome atlas UCEC (uterine corpus endometrioid carcinoma) RNA sequencing data set.¹⁴

Paired end raw reads (fastq format) were quality- and adapter-filtered using the Trim-galore wrapper for FastQC and cutadapt (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). The retained paired reads were aligned to the Ensembl annotated HG19 human Illumina iGenome build using Tophat2, and differential gene expression was calculated for PCOS and EC specimens relative to the control specimen using Cuffdiff¹⁵ on the basis of fold changes >1.5 and P-value <.05. Statistically significantly enriched gene ontologies and pathways for differentially expressed genes were obtained using WebGestalt and the Cytoscape Genemania plugin.¹⁶ Next-generation RNAseq and associated clinical information was obtained from the cancer genome atlas endometrium cancer (UCEC) data set.¹⁴ Normalized RSEM expression counts scaled to library size from each patient were compiled and correlated with specific clinical features including tumour and nontumour endometrial tissue and grade were analysed using EdgeR¹⁷ or the Wilcoxon test with Benjamini-Hochberg false discovery rate correction for multiple testing.

We used qRT-PCR to validate differential endometrial gene expression identified by RNAseq in a subset of Nottingham cohort of PCOS, EC and control patients for whom mRNA and cDNA were available as previously described.¹⁰ The hydrolysis probe PCR reagents employed

were as follows: β -actin: Hs01060665_g1; NQO1: Hs02512143_s1. Each sample was analysed in triplicate using the Plaffl method. For qPCR experiments, unpaired *t* tests were used to compare expression between control, PCOS and EC specimens.

2.3 | Immunohistochemistry

Samples from a separate cohort at the University of Manchester investigating prognostic biomarkers in EC using immunohistochemistry provided an opportunity to further investigate and validate the role of differentially expressed genes in EC. The Manchester cohort consisted of consecutive patients (*n* = 91) who underwent hysterectomy for EC at St Mary's Hospital in Manchester between 2011 and 2013, and who provided written, informed consent for their tumour samples to be stored in the BRC Biobank and used for future research. A further 6 postmenopausal women with histologically normal endometrium who underwent hysterectomy for genital prolapse were also included. The study received ethical approval from NRES Committee London - Fulham (REC reference 12/LO/0364) and R&D approval (R01960) from Central Manchester University Hospitals NHS Foundation Trust. The EC cohort comprised different histological subtypes, grades and stages of EC that were fully annotated with respect to patient demographics and clinical follow-up data. The average follow-up for the Manchester cohort was 34 months (range 1-64), during which time there were 19 recurrences and 23 deaths, of which 13 were EC-specific.

Formalin-fixed, paraffin-embedded tissue samples were cut into 4- μ m sections for IHC analysis. This was performed using a fully automated IHC platform, Leica BOND-MAX together with Bond™ Polymer Refine Detection kit (DS9800) and on-board retrieval system. The sections were labelled with NQO1 (Sigma, 1:75 dilution) primary antibody according to standard validated Protocol F written by Leica. The detection kit was a biotin-free, polymeric horseradish peroxidase (HRP)-linker antibody conjugate system that detects tissue-bound IgG primary antibodies using the chromogen 3,3'-diaminobenzidine tetrahydrochloride hydrate (DAB) via a brown precipitate. Tissue sections were then counterstained with haematoxylin. Immunohistochemical evaluation was performed blindly by two independent observers (AL and AC) and discordant cases settled by review. NQO-1 staining was scored using the product of the area score (proportion of positively staining tumour cells) and the intensity of staining (0-3, 0 = zero staining, 3 = high-intensity staining). The score range was 0-300, and tumours were then dichotomised into low expression (score < 200) and high expression (score > 200).

2.4 | Statistical analysis

NQO1 protein expression in normal and malignant endometrium was compared using the Mann-Whitney *U* test. The association between NQO1 protein expression and clinical-pathological variables in women with endometrial cancer was tested using the Mann-Whitney *U* test for nonparametric variables and Spearman rank correlation for continuous and ordinal variables. Kaplan-Meier curves were constructed to estimate the effect of NQO1 expression on overall, cancer-specific and recurrence-free survival, with curves compared using the log-rank

test. Overall survival was defined as the time between date of surgery and death from any cause, while cancer-specific survival referred to the time interval between surgery and death from endometrial cancer. Recurrence-free survival was defined as the time between date of surgery and first documented local or distant recurrence. Data without events were censored at date of last clinical follow-up visit. A Cox proportional hazard regression model was used in a univariate analysis of cancer-specific and recurrence-free survival, after confirming that the data were complied with the proportional hazards assumption using log-log curves. All clinical-pathological variables with known prognostic value in endometrial cancer were included in the univariate analysis alongside NQO1.

3 | RESULTS

3.1 | Patient demographics for samples used for the RNA sequencing and PCR validation study

Samples from three women were submitted for RNA sequencing. One patient with EC (BMI = 35.9, age 43), one PCOS patient without EC (BMI = 35.9, age 41) and an unaffected control woman (BMI = 32, age 42) were used for the RNA sequencing experiments. RNAseq identified differentially expressed genes (using standard criteria of fold changes [FC] >1.5, *P*-value <.05) in PCOS (700 genes) and EC (776 genes) endometrial specimens relative to control nonmalignant endometrium (Figure 1A-D, Tables S1, S2). We found that the global transcriptional profile of endometrial tissue from the woman with PCOS was most similar to the control obese woman (Figure 1). Of these genes, 94 genes were differentially expressed in both EC and PCOS relative to control endometrium (Table S1). Specifically, 12 genes were higher and 82 were lower in PCOS and EC specimens relative to control endometrium (Table S1, S2).

In the qRT-PCR validation cohort, the BMIs of women with EC ($33.12 \pm 5.959 \text{ kg/m}^2$), PCOS ($31.88 \pm 5.975 \text{ kg/m}^2$) and controls ($29.27 \pm 2.467 \text{ kg/m}^2$) were not significantly different. PCOS women were however younger (31.88 ± 5.975 years) than women with EC (62.64 ± 11.10 years) and controls (45.96 ± 13.34 years). Women with PCOS were recruited during their proliferative menstrual phase (based on their menstrual histories). We next used qRT-PCR to validate the expression of a subset of these genes in our patient cohorts (Figure 1E, Figure S1B). We confirmed expression of NQO1 (Figure 1E), the NQO1 target *p53* and another exemplar gene identified by RNAseq, *GJB2*, (Figure S1) was significantly increased (*P* < .05) in endometrial specimens from women with PCOS (*n* = 25) and EC (*n* = 25) as compared to control, unaffected women (*n* = 25). We next examined expression of these 94 genes in patients with EC using the cancer genome atlas.¹⁷ Of these 94 genes, 14 genes (*NQO1*, *SLPI*, *GJB2*, *DNAJC15*, *S100A8*, *PLEKHS1*, *ESPN*, *RSPH1*, *KRT5*, *FOXJ1*, *IFI27*, *IFI6*, *LGR5* and *MUC16*) were significantly altered in tumour as compared to nontumour endometrial specimens (Figure S1). Expression of NQO1 and its target *p53* mRNA are all significantly higher in primary endometrial tumour (*n* = 370) than nontumour (*n* = 11) specimens (Figure S1) as reported in the cancer genome atlas UCEC data set. The Genemania Cytoscape plugin was used

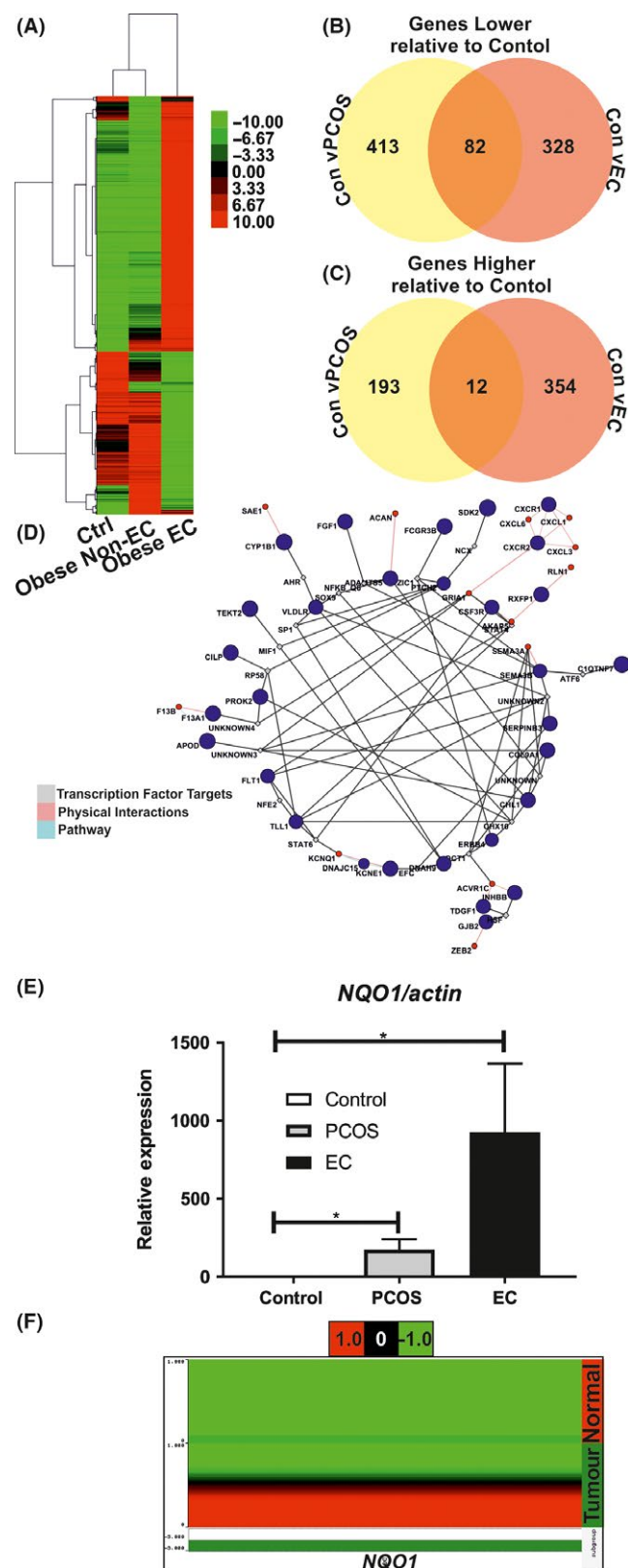


FIGURE 1 Next-generation RNA sequencing was used to compare the transcriptome of endometrial samples from unaffected, PCOS and EC patients. Unsupervised hierarchical clustering indicates nonmalignant endometrial specimens from unaffected control and PCOS patients are most similar. Elevated gene expression is indicated in red, and lower gene expression is indicated in green (A). A subset of 94 genes comprised of 82 down-regulated genes and 12 upregulated (B, C) are commonly dysregulated in PCOS and malignant endometrium. The Genemania cytoscape plugin was used to identify common pathways and infer potential transcriptional regulators of the gene network (D). We used qRT-PCR (E) and the cancer genome atlas (F) to compare expression of *NQO1* normalized to actin in endometrial biopsies from PCOS ($n = 25$) and EC ($n = 25$) women relative to unaffected women ($n = 25$). **** = $P < .005$ by Kruskal-Wallis nonparametric analysis of variance with Dunn's post hoc multiple comparisons test. We also analysed expression of *NQO1*, and its downstream target *p53* in the cancer genome atlas UCEC data set ($N = 370$ EC, $N = 11$ nonmalignant endometrium). Expression of *NQO1* is significantly elevated in tumour, relative to nonmalignant endometrial tissue as determined by Wilcoxon test with Benjamini-Hochberg false discovery rate correction for multiple testing. [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | Immunohistochemistry validating the role of NQO1 in EC

The Manchester EC patient demographics and clinicopathological features are shown (Table 2). The control women were postmenopausal with histologically normal endometrium and underwent hysterectomy for genital prolapse. Histologically normal postmenopausal endometrium did not express *NQO1* (Figure 2). In EC, there was a statistically significant association between high *NQO1* expression and advancing age (Table S3). There was no statistically significant correlation between *NQO1* expression and standard clinicopathological features with established prognostic value, including histological subtype, grade or stage of disease, deep myometrial invasion or LVSI (Table S3). Both type 1 (endometrioid) and type 2 (nonendometrioid) EC expressed *NQO1*, and there was a trend towards poorer outcomes with higher *NQO1* staining; however, *NQO1* expression was not associated with recurrence-free, EC-specific or overall survival in the Manchester cohort (Table S3, Figure S2). This may reflect the good overall prognosis of EC and an insufficient number of events to demonstrate significance.

4 | DISCUSSION

Known risk factors for EC include increasing age, polycystic ovary syndrome (PCOS), obesity and type 2 diabetes.¹⁸⁻²¹ We and others have identified altered *SREBP1*,¹¹ and insulin signalling in endometrial specimens from women with PCOS or EC.^{10,22} Anovulatory menstrual cycles, commonly found in PCOS women, have also been linked with EC.²³ The mechanisms are thought to involve a state of progesterone deficiency. Progesterone protects the endometrium from the mitogenic effects of oestrogen and withdrawal of progesterone triggers endometrial sloughing (menstruation), which allows

to identify pathways and infer potential transcriptional regulators of the genes identified common to PCOS and EC (Figure 1D). Enriched gene ontologies defined by these differential genes were identified (Table S2). Interestingly, the significantly enriched gene ontologies included gene networks involved in microtubule motor activity and cilia function.

Characteristic	NQO1			P value
	All n = 91	NQO1 score <200 n = 53	NQO1 score ≥200 n = 38	
Median age at diagnosis years (IQR)	68 (58-74)	67 (56-72)	72.5 (63.3-77.8)	.007**
Median BMI at diagnosis kg/m ² (IQR)	30.1 (26.1-37.1)	30.1 (26.0-39.1)	29.6 (26.3-35.2)	.622
Diabetic, n (%)				
No	71 (78.0)	43 (81.1)	28 (73.7)	.629
Yes	20 (22.0)	10 (18.9)	10 (26.3)	
Histological grade, n (%)				
1	23 (25.3)	15 (28.3)	8 (21.1)	.164
2	20 (22.0)	12 (22.6)	8 (21.1)	
3	48 (52.7)	26 (49.1)	22 (57.9)	
FIGO (2009) stage, n (%)				
1	59 (64.8)	34 (64.2)	25 (65.8)	.115
2	12 (13.2)	9 (17.0)	3 (7.9)	
3	18 (19.8)	9 (17.0)	9 (23.7)	
4	2 (2.2)	1 (1.9)	1 (2.6)	
Histological type, n (%)				
Endometrioid	48 (52.7)	31 (58.4)	17 (44.7)	.100
Nonendometrioid	43 (47.3)	22 (41.5)	21 (55.3)	
Lymphovascular space invasion, n (%)				
Absent	50 (53.8)	32 (60.4)	18 (47.4)	.356
Present	38 (41.8)	18 (34.0)	20 (52.6)	
Missing data	3 (3.3)	3 (5.7)	0 (0)	
Depth of myometrial invasion, n (%)				
<50%	51 (56.0)	31 (58.5)	20 (52.6)	.412
≥ 50%	40 (44.0)	22 (41.5)	18 (47.4)	
Any adjuvant treatment, n (%)				
No	37 (40.7)	23 (43.4)	14 (36.8)	.366
Yes	54 (59.3)	30 (56.6)	24 (63.2)	

**indicates *p* values <0.01.

TABLE 2 Relationship between known prognostic variables and NQO1 expression in the Manchester cohort

the natural shedding of abnormal endometrial cells. A common systemic pathway such as an aberrant insulin signalling pathway may cause oligo/amenorrhoea as well as endometrial hyperplasia and EC independent of body mass index (BMI). In such a scenario, aberrant systemic signalling programs pro-oncogenic transcriptional networks in the endometrium of women with PCOS that may predispose to EC. Indeed, while previous gene expression studies have investigated PCOS²⁴ and EC,²⁵ the exact mechanisms that predispose obese women to EC are unclear. Thus, while the epidemiological evidence supporting an association between PCOS and an increased risk of endometrial carcinogenesis is robust,^{10,19-21} a definitive mechanistic link between the conditions has yet to be identified. For this reason, the goal of this study was to compare endometrial gene expression profiles from women with endometrial cancer and PCOS. The current study is the first to compare global gene expression in

endometrial specimens from women with PCOS and EC. Our findings have identified 94 genes, including NQO1, commonly altered in endometrial specimens from women with PCOS and EC, suggesting a potential common mechanism in the disorders.

NQO1 has an established role in the endometrium.²⁶ NQO1 encodes NAD(P)H:quinone oxidoreductase 1 in detoxification pathways²⁷⁻²⁹ and has been reported to activate specific quinone-derived pharmaceuticals including mitomycin C and apaziquone.^{30,31} NQO1 also acts to protect the p53 tumour suppressor protein, and many other proteins involved in proliferation from proteasomal degradation.³² Interestingly, missense variants in NQO1 are implicated in many cancer types³³⁻³⁵ and more recently, increased NQO1 expression is associated with poor prognosis in ovarian³⁶ and lung³⁷ cancers. There is also considerable interest in NQO1 as a cancer therapeutic target. Its ability to activate cytotoxic therapies

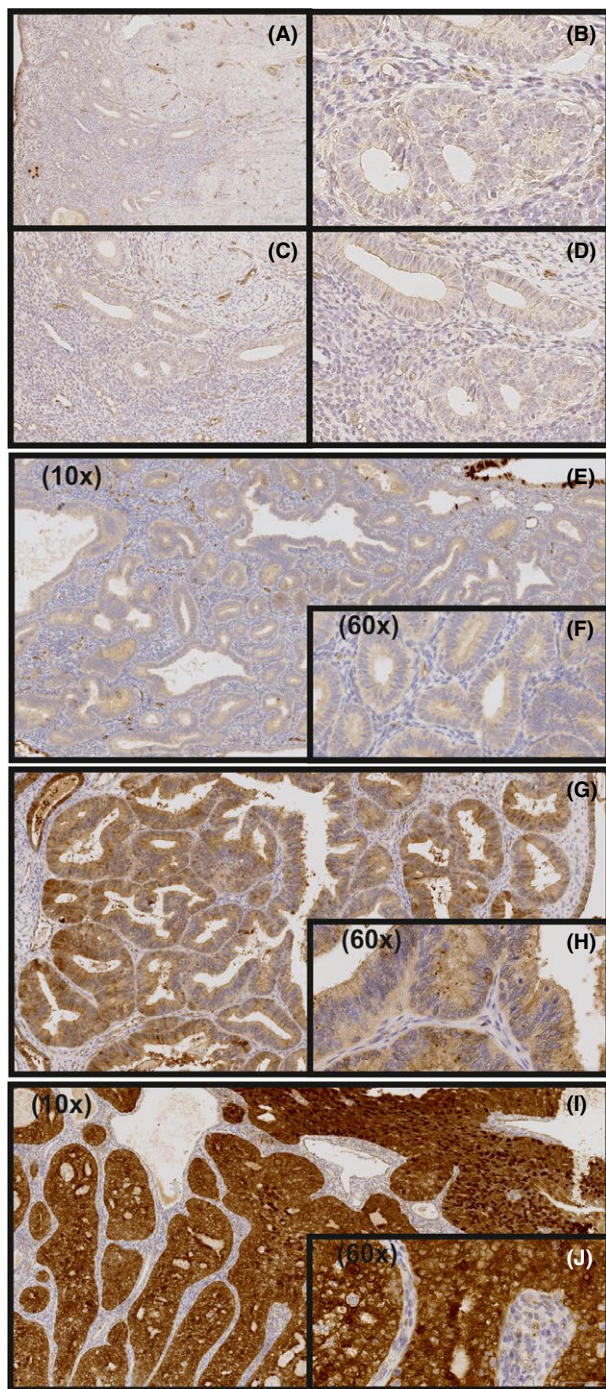


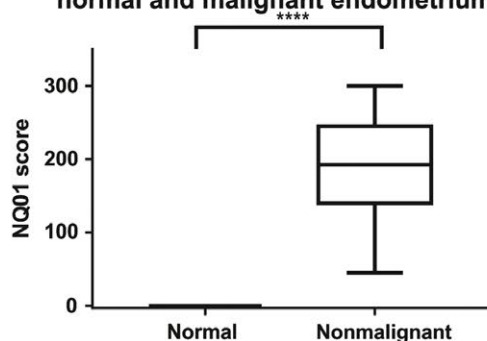
FIGURE 2 Immunohistochemistry was used to evaluate expression of NQO1 protein in nontumour (A-D) and endometrial cancer specimens (E-J). Representative NQO1 staining of endometrial cancer specimens with intensity scores of 1 (E, F), 2 (G, H) and 3 (I, J) at 10 \times and 60 \times magnification is shown. (F) Comparison of NQO1 expression in normal and malignant endometrium as determined by immunohistochemistry. NQO1 was expressed solely in endometrial cancers and not demonstrated in normal endometrium from control patients ($P < .0001$). [Colour figure can be viewed at wileyonlinelibrary.com]

selectively within malignant tissue may be an attractive therapeutic approach.³⁸ Consistent with this NQO1 null mice are more sensitive to chemical induced carcinogenesis³² and NQO1 plays an essential role in oncogene-induced senescence. However, the association of overexpression of NQO1 with poorer outcomes in certain cancer types^{36,37} suggests that tumours can bypass the antiproliferative actions of NQO1, potentially through the activation of pro-carcinogenic compounds.³⁹

In this current study, expression of NQO1 was significantly higher in tumour as compared to matched nontumour specimens (Figures 1, 2). NQO1 expression is regulated by the oestrogen receptor- α (ER α /NR3A1) and oestrogen receptor- β (ER β /NR3A2)⁴⁰ and by progesterone.⁴¹ As outlined above, aberrant oestrogen and progesterone signalling contributes to EC risk. Related to this, the antibreast cancer selective oestrogen receptor modulator, tamoxifen, is known to increase EC risk by inducing oestrogen-regulated gene expression⁴² and altering oestrogen metabolism in endometrial cells.⁴³ NQO1 may therefore play a key role in the oestrogen-related links between EC and PCOS.

In conclusion, we have used a preliminary RNAseq analysis to identify aberrant gene expression in EC and endometrium from women with PCOS. One limitation to our study was that only individual patient samples were sequenced. However, we confirmed expression of a key gene identified by RNAseq, NQO1, in larger cohorts of patients with PCOS and EC at the mRNA and protein levels. In this study, increased NQO1 expression was not associated with standard prognostic clinicopathological features including tumour type, grade, stage and myometrial invasion lymphovascular space invasion (LVSI). Indeed, NQO1 was expressed ubiquitously in most tumours and was not associated with recurrence-free, endometrial cancer-specific or overall survival. However, NQO1 expression, like that of 93 other transcripts, was deregulated in both PCOS and EC. This supports the hypothesis that PCOS can induce gene expression changes in the endometrium that resembles EC. It is possible such changes in gene expression contribute to the increased risk of EC in women with PCOS. NQO1 represents a potential therapeutic target in EC. NQO1 is inhibited by dicoumarol, and more specific next-generation NQO1-inhibitors are now available (Figure 3).⁴⁴ Therefore, the preclinical testing of such compounds for the treatment of EC and prevention of future EC in PCOS is warranted. Finally, further studies are now warranted to examine NQO1 protein expression in endometrial biopsies from women with PCOS in a prospective study to determine whether NQO1 may be useful to distinguish women at increased risk of developing EC.

(K) Comparison of NQO1 expression in normal and malignant endometrium



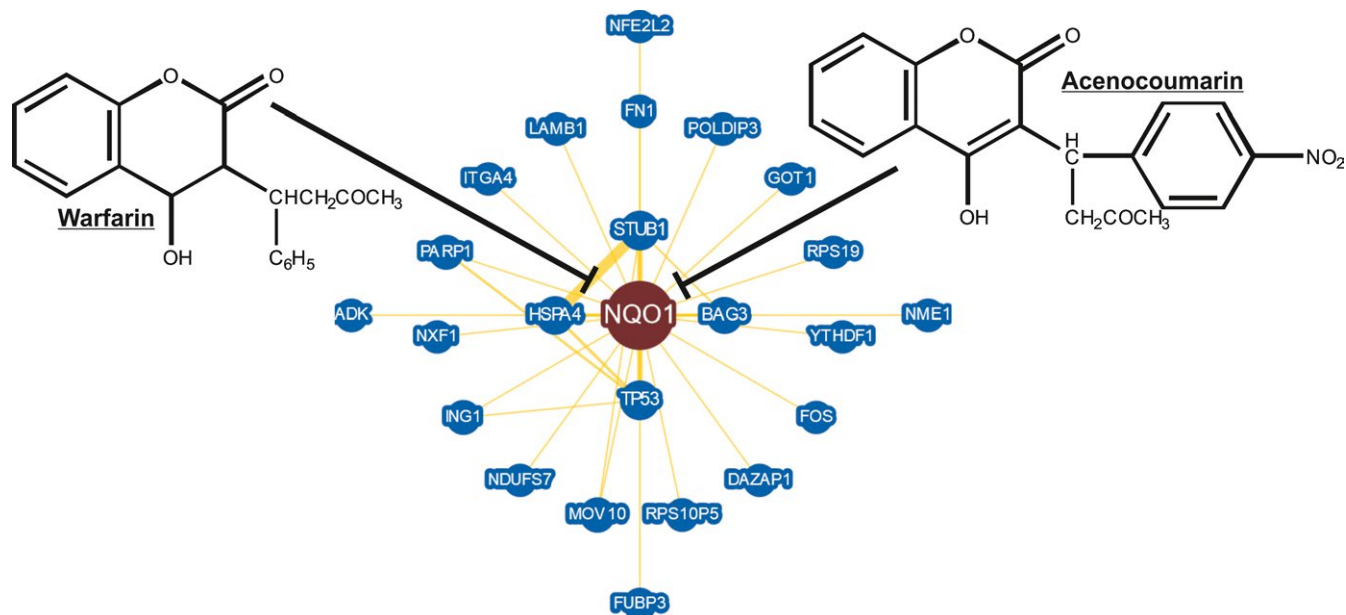


FIGURE 3 The BioGRID database⁴⁵ reports NQO1 functionally interacts with key regulators of cell proliferation including TP53. Furthermore, the enzymatic activity of NQO1 can be inhibited by the anticoagulants, warfarin and acenocoumarin and more specific next-generation NQO1-inhibitors are now available. This may point to potential chemo-preventative approaches to pharmacologically target elevated expression of NQO1 function in women with PCOS. [Colour figure can be viewed at wileyonlinelibrary.com]

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CONFLICT OF INTERESTS

The authors confirm no conflict of interests related to this study.

AUTHOR CONTRIBUTIONS

WA, EJC, MNS and NPM involved in study design, patient recruitment and project management. MNS, CC, VMM, JA, AL, AC, SK, VS, IS, CSR, JLP, NO, PF-U, DMH, CSR, NPM, EJC and WA conducted the

experiments and data analysis. WA, EJC, JA, VNS, AL, NO, JLP, JJ, CSR, IJS, DMH and NPM wrote the manuscript.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016;66:7-30.
2. Crosbie EJ, Zwahlen M, Kitchener HC, Egger M, Renehan AG. Body mass index, hormone replacement therapy, and endometrial cancer risk: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2010;19:3119-3130.
3. Crosbie E, Morrison J. The emerging epidemic of endometrial cancer: time to take action. *Cochrane Database Syst Rev* 2014;12:ED000095. <https://doi.org/10.1002/14651858.ED000095>
4. Hickey M, Doherty DA, Atkinson H, et al. Clinical, ultrasound and biochemical features of polycystic ovary syndrome in adolescents: implications for diagnosis. *Hum Reprod*. 2011;26:1469-1477.
5. Yildiz BO, Bozdag G, Yapici Z, Esinler I, Yarali H. Prevalence, phenotype and cardiometabolic risk of polycystic ovary syndrome under different diagnostic criteria. *Hum Reprod*. 2012;27:3067-3073.
6. Peigne M, Dewailly D. Long term complications of polycystic ovary syndrome (PCOS). *Ann Endocrinol (Paris)*. 2014;75:194-199.
7. Haoula Z, Salman M, Atiomo W. Evaluating the association between endometrial cancer and polycystic ovary syndrome. *Hum Reprod*. 2012;27:1327-1331.
8. Barry JA, Azizia MM, Hardiman PJ. Risk of endometrial, ovarian and breast cancer in women with polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod Update*. 2014;20:748-758.
9. Shafiee MN, Chapman C, Barrett D, Abu J, Atiomo W. Reviewing the molecular mechanisms which increase endometrial cancer (EC) risk in women with polycystic ovarian syndrome (PCOS): time for paradigm shift? *Gynecol Oncol*. 2013;131:489-492.
10. Shafiee MN, Seedhouse C, Mongan N, et al. Up-regulation of genes involved in the insulin signalling pathway (IGF1, PTEN and IGFBP1) in

- the endometrium may link polycystic ovarian syndrome and endometrial cancer. *Mol Cell Endocrinol*. 2016;424:94-101.
11. Shafiee MN, Mongan N, Seedhouse C, et al. Sterol regulatory element binding protein-1 (SREBP1) gene expression is similarly increased in polycystic ovary syndrome and endometrial cancer. *Acta Obstet Gynecol Scand*. 2017;96:556-562.
 12. Galazis N, Pang YL, Galazi M, Haoula Z, Layfield R, Atiomo W. Proteomic biomarkers of endometrial cancer risk in women with polycystic ovary syndrome: a systematic review and biomarker database integration. *Gynecol Endocrinol*. 2013;29:638-644.
 13. Piltonen TT, Chen JC, Khatun M, et al. Endometrial stromal fibroblasts from women with polycystic ovary syndrome have impaired progesterone-mediated decidualization, aberrant cytokine profiles and promote enhanced immune cell migration in vitro. *Hum Reprod*. 2015;30:1203-1215.
 14. Kandoth C, Schultz N, Cherniack AD, et al. Integrated genomic characterization of endometrial carcinoma. *Nature*. 2013;497:67-73.
 15. Trapnell C, Roberts A, Goff L, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat Protoc*. 2012;7:562-578.
 16. Montojo J, Zuberi K, Rodriguez H, Bader GD, Morris Q. GeneMANIA: fast gene network construction and function prediction for Cytoscape. *F1000Res* 2014;3:153.
 17. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2010;26:139-140.
 18. Schmandt RE, Iglesias DA, Co NN, Lu KH. Understanding obesity and endometrial cancer risk: opportunities for prevention. *Am J Obstet Gynecol*. 2011;205:518-525.
 19. Husing A, Dossus L, Ferrari P, et al. An epidemiological model for prediction of endometrial cancer risk in Europe. *Eur J Epidemiol*. 2016;31:51-60.
 20. Wild S, Pierpoint T, Jacobs H, McKeigue P. Long-term consequences of polycystic ovary syndrome: results of a 31 year follow-up study. *Hum Fertil (Camb)*. 2000;3:101-105.
 21. Fearnley EJ, Marquart L, Spurdle AB, Weinstein P, Webb PM, Australian Ovarian Cancer Study, G, Australian National Endometrial Cancer Study, G. Polycystic ovary syndrome increases the risk of endometrial cancer in women aged less than 50 years: an Australian case-control study. *Cancer Causes Control*. 2010;21:2303-2308.
 22. Shafiee MN, Khan G, Ariffin R, et al. Preventing endometrial cancer risk in polycystic ovarian syndrome (PCOS) women: could metformin help? *Gynecol Oncol*. 2014;132:248-253.
 23. Schindler AE. Progestogen deficiency and endometrial cancer risk. *Maturitas*. 2009;62:334-337.
 24. Jansen E, Laven JS, Dommerholt HB, et al. Abnormal gene expression profiles in human ovaries from polycystic ovary syndrome patients. *Mol Endocrinol*. 2004;18:3050-3063.
 25. Zorn KK, Bonome T, Gangi L, et al. Gene expression profiles of serous, endometrioid, and clear cell subtypes of ovarian and endometrial cancer. *Clin Cancer Res*. 2005;11:6422-6430.
 26. Hevir N, Ribic-Pucelj M, Lanisnik Rizner T. Disturbed balance between phase I and II metabolizing enzymes in ovarian endometriosis: a source of excessive hydroxy-estrogens and ROS? *Mol Cell Endocrinol*. 2013;367:74-84.
 27. Asher G, Lotem J, Kama R, Sachs L, Shaul Y. NQO1 stabilizes p53 through a distinct pathway. *Proc Natl Acad Sci U S A*. 2002;99:3099-3104.
 28. Ross D, Siegel D. NAD(P)H:quinone oxidoreductase 1 (NQO1, DT-diaphorase), functions and pharmacogenetics. *Methods Enzymol*. 2004;382:115-144.
 29. Talalay P, Dinkova-Kostova AT. Role of nicotinamide quinone oxidoreductase 1 (NQO1) in protection against toxicity of electrophiles and reactive oxygen intermediates. *Methods Enzymol*. 2004;382:355-364.
 30. Phillips RM, Hendriks HR, Peters GJ, Pharmacology E, Molecular Mechanism G. EO9 (Apaziquone): from the clinic to the laboratory and back again. *Br J Pharmacol*. 2013;168:11-18.
 31. Siegel D, Beall H, Kasai M, Arai H, Gibson NW, Ross D. pH-dependent inactivation of DT-diaphorase by mitomycin C and porfiromycin. *Mol Pharmacol*. 1993;44:1128-1134.
 32. Iskander K, Gaikwad A, Paquet M, et al. Lower induction of p53 and decreased apoptosis in NQO1-null mice lead to increased sensitivity to chemical-induced skin carcinogenesis. *Cancer Res*. 2005;65:2054-2058.
 33. Lajin B, Alachkar A. The NQO1 polymorphism C609T (Pro187Ser) and cancer susceptibility: a comprehensive meta-analysis. *Br J Cancer*. 2013;109:1325-1337.
 34. Fagerholm R, Hofstetter B, Tommiska J, et al. NAD(P)H:quinone oxidoreductase 1 NQO1*2 genotype (P187S) is a strong prognostic and predictive factor in breast cancer. *Nat Genet*. 2008;40:844-853.
 35. Jamshidi M, Bartkova J, Greco D, et al. NQO1 expression correlates inversely with NFkappaB activation in human breast cancer. *Breast Cancer Res Treat*. 2012;132:955-968.
 36. Cui X, Li L, Yan G, et al. High expression of NQO1 is associated with poor prognosis in serous ovarian carcinoma. *BMC Cancer*. 2015;15:244.
 37. Li Z, Zhang Y, Jin T, et al. NQO1 protein expression predicts poor prognosis of non-small cell lung cancers. *BMC Cancer*. 2015;15:207.
 38. Bey EA, Bente MS, Reinicke KE, et al. An NQO1- and PARP-1-mediated cell death pathway induced in non-small-cell lung cancer cells by beta-lapachone. *Proc Natl Acad Sci U S A*. 2007;104:11832-11837.
 39. Martin FL, Patel II, Sozeri O, et al. Constitutive expression of bioactivating enzymes in normal human prostate suggests a capability to activate pro-carcinogens to DNA-damaging metabolites. *Prostate*. 2010;70:1586-1599.
 40. Montano MM, Jaiswal AK, Katzenellenbogen BS. Transcriptional regulation of the human quinone reductase gene by antiestrogen-liganded estrogen receptor-alpha and estrogen receptor-beta. *J Biol Chem*. 1998;273:25443-25449.
 41. Morrissy S, Strom J, Purdom-Dickinson S, Chen QM. NAD(P)H:quinone oxidoreductase 1 is induced by progesterone in cardiomyocytes. *Cardiovasc Toxicol*. 2012;12:108-114.
 42. Shang Y, Brown M. Molecular determinants for the tissue specificity of SERMs. *Science*. 2002;295:2465-2468.
 43. Williams-Brown MY, Salih SM, Xu X, et al. The effect of tamoxifen and raloxifene on estrogen metabolism and endometrial cancer risk. *J Steroid Biochem Mol Biol*. 2011;126:78-86.
 44. Scott KA, Barnes J, Whitehead RC, Stratford IJ, Nolan KA. Inhibitors of NQO1: identification of compounds more potent than dicoumarol without associated off-target effects. *Biochem Pharmacol*. 2011;81:355-363.
 45. Chatr-Aryamontri A, Breitkreutz BJ, Oughtred R, et al. The BioGRID interaction database: 2015 update. *Nucleic Acids Res*. 2015;43:D470-D478.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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